Reconstruction of the Phenotypes of Methicillin-Resistant *Staphylococcus aureus* by Replacement of the Staphylococcal Cassette Chromosome *mec* with a Plasmid-Borne Copy of *Staphylococcus sciuri pbpD* Gene[†]

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The *mecA* gene, the central determinant of methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA), is not native to this bacterial species but may have originated in the animal commensal species *Staphylococcus sciuri*. All *S. sciuri* strains carry a close homologue of *mecA* in the form of *pbpD*, the genetic determinant of penicillin binding protein 4 (PBP 4) of *S. sciuri*. Here we describe an experimental system that could be used for additional tests for this proposition. The *S. sciuri pbpD* gene was cloned into a shuttle plasmid and introduced into methicillin-susceptible *S. aureus* strain COL-S derived from parental MRSA strain COL from which the resistance cassette staphylococcal cassette chromosome *mec* was excised. The *S. sciuri pbpD* determinant was transcribed and translated in the *S. aureus* transductants producing large amounts of the 84-kDa *S. sciuri* PBP 4 and was then deposited in the plasma membrane of the host bacterium. Transductants carrying the heterologous *S. sciuri pbpD* gene exhibited properties typical of those of parental MRSA strain COL, including broad-spectrum, high-level, and homogeneous resistance to structurally different β -lactams. Antibiotic resistance was dependent on the functioning of *S. aureus* PBP 2 and was suppressed by the specific regulatory genes *mecI* and *mecR* and by inhibitors of an early step in cell wall biosynthesis. *S. sciuri* PBP 4 was also able to replace the essential physiological function(s) of the native PBP 2 of *S. aureus* and produce peptidoglycan typical of that of parental MRSA strain COL. Our results provide further support for the proposition that the resistance determinant *mecA* of MRSA strains has evolved from *S. sciuri pbpD*.

Staphylococcus aureus is a major human pathogen responsible for several life-threatening infections, including septicemia, endocarditis, and toxic shock syndrome. Methicillin (meticillin)-resistant *S. aureus* (MRSA) strains were first reported in 1961, shortly after the introduction of methicillin in clinical practice. Since then, MRSA had become an increasing critical threat in hospital and community environments worldwide. The genetic determinant of β -lactam resistance, the *mecA* gene, is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*), and encodes a low-affinity penicillin binding protein (PBP), PBP 2A (8). Several lines of evidence suggest that the heterologous β -lactam resistance gene, *mecA*, which is resident in all MRSA strains, may have its evolutionary origin in a close homologue of this gene that is ubiquitous in both β -lactam-susceptible and -resistant isolates of the animal commensal species *Staphylococcus sciuri* (2, 3). The *S. sciuri pbpD* gene (the *S. aureus mecA* homologue) is the genetic determinant of PBP 4, one of the six PBPs recently identified in this species (20). Previous studies have shown that an upregulated form of the *S. sciuri pbpD* gene transduced into a susceptible *S. aureus* strain was able to produce a moderate but significant (twofold) increase in the oxacillin MIC of the transductants (19, 20). In that experiment, the source of the upregulated *pbpD* gene was the laboratory mutant K1M200, ob-

tained by stepwise exposure of antibiotic-susceptible *S. sciuri* strain K1 to gradually increasing concentrations of methicillin. The recipient strain was *S. aureus* COL, in which the *mecA* resistance determinant was inactivated by a transposon insert.

The purpose of the studies described here was to construct a new experimental system that would allow one to further test the validity of the proposition that the resistance determinant of MRSA strains may have originated from the *S. sciuri pbpD* gene. The chromosomal resistance determinant SCC*mec* was excised from highly and homogeneously methicillin-resistant *S. aureus* strain COL to provide a methicillin-susceptible *S. aureus* (MSSA) strain, COL-S. In some of the new experiments, the essential *S. aureus pbpB* gene was also put under the control of an inducible promoter producing strain, COL-S_{pac::pbpB}. Both strains were then used as the recipient for plasmid-borne copies of the upregulated *S. sciuri pbpD* gene recovered from oxacillin-resistant clinical isolate SS37 of *S. sciuri* (3, 4). Transductants carrying the *S. aureus mecA* determinant on the same plasmid were used as controls. The physiological, genetic, and biochemical properties of the *S. aureus* COL-S transductants carrying the heterologous *S. sciuri pbpD* gene were then compared to the properties of original MRSA strain COL to determine to what extent the drug resistance-related phenotypes of the original MRSA strain were reconstructed in the transductants.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The characteristics of the bacterial strains and plasmids used in this study are described in Table 1. Bac-

TABLE 1. Bacterial strains and plasmids^a

Strain or plasmid	Characteristics	Source or reference
<i>S. aureus</i> strains		
COL	Clinical isolate; Ox ^r Em ^s Cm ^s Tc ^r	RU collection
COL _{spac::pbpB}	COL derivative in which <i>pbpB</i> is under the control of <i>spac</i> promoter/pPBP2i; Em ^r	13
COL-S	COL with SCCmec excised; Ox ^s Em ^s Cm ^s Tc ^s	11
COL-S _{spac::pbpB}	COL-S derivative in which <i>pbpB</i> is under the control of <i>spac</i> promoter/pPBP2i; Em ^r	This study
COL-S _{AmecA}	COL-S carrying the plasmid-borne <i>S. aureus mecA</i> region/pSTSW2C; Tc ^r	This study
COL-S _{SSpbpD}	COL-S carrying the plasmid-borne <i>S. sciuri pbpD</i> region/pSS37MA; Tc ^r	This study
COL-S _{spac::pbpB/SAmecA}	COL-S _{spac::pbpB} carrying the plasmid-borne <i>S. aureus mecA</i> region/pSTSW2C; Em ^r Tc ^r	This study
COL-S _{spac::pbpB/SSpbpD}	COL-S _{spac::pbpB} carrying the plasmid-borne <i>S. sciuri pbpD</i> region/pSS37MA; Em ^r Tc ^r	This study
COL _{mecI-mecR1}	COL carrying the plasmid-borne <i>S. aureus mecI-mecR1</i> region/pGC2:: <i>mecI-mecR1</i> ; Cm ^r	D. C. Oliveira
COL-S _{AmecA/mecI-mecR1}	COL-S _{AmecA} carrying the plasmid-borne <i>S. aureus mecI-mecR1</i> region/pGC2:: <i>mecI-mecR1</i> ; Tc ^r Cm ^r	This study
COL-S _{SSpbpD/mecI-mecR1}	COL-S _{SSpbpD} carrying the plasmid-borne <i>S. aureus mecI-mecR1</i> region/pGC2:: <i>mecI-mecR1</i> ; Tc ^r Cm ^r	This study
<i>S. sciuri</i> strain SS37	Clinical isolate recovered from the nasopharynx of a patient; Ox ^r	4
Plasmids		
pPBP2i	<i>S. aureus</i> integrational vector with <i>pbpB</i> ribosome-binding site and first 171 codons fused to P _{spac} promoter; Em ^r	13
pSTSW2C	pSPT181C with 3,737-bp PCR product of <i>S. aureus</i> COL <i>mecA</i> region; Tc ^r	19
pSS37MA	pSPT181C with 4,130-bp PCR product of <i>S. sciuri</i> SS37 <i>pbpD</i> region; Tc ^r	20
pGC2:: <i>mecI-mecR1</i>	pGC2 with 2,466-bp PCR product of <i>S. aureus</i> N315 <i>mecI-mecR1</i> region; Cm ^r	D. C. Oliveira

^a Abbreviations: Ox^s, oxacillin susceptible; Ox^r, oxacillin resistant; Em^s, erythromycin susceptible; Em^r, erythromycin resistant; Cm^s, chloramphenicol susceptible; Cm^r, chloramphenicol resistant; Tc^s, tetracycline susceptible; Tc^r, tetracycline resistant; RU, The Rockefeller University.

terial cultures were grown in tryptic soy broth (Difco Laboratories) or on tryptic soy agar (Difco Laboratories) at 30°C with aeration.

Antibiotic susceptibility testing. The susceptibilities of the *S. aureus* strains to β -lactam antibiotics were determined by population analysis, as described previously (17).

Peptidoglycan purification and analysis by HPLC. Peptidoglycan was purified from 1-liter cultures of bacteria grown at 30°C to mid-exponential phase, as described previously (5). Purified peptidoglycan was digested with mutanolysin (Sigma-Aldrich); and mucopeptides were reduced with sodium borohydride (Sigma-Aldrich), separated by high-performance liquid chromatography (HPLC) on a C₁₈ column (3 μ m, 4.6 by 250 mm; ODS-Hypersil; Thermo Electron Corporation), and detected by measurement of the absorbance at 206 nm, as described previously (5).

Membrane purification and PBP assay. Membrane preparations were purified from cultures grown to the late exponential phase, as described previously (15). The detection of PBPs was performed by incubating the membrane preparations (150 μ g of proteins) with a saturating concentration (20 μ g/ml) of [¹⁴C]benzylpenicillin (59 mCi/mmol, 158 μ Ci/mg; GE Healthcare) at 30°C for 10 min. The reactions were stopped by adding an excess of unlabeled penicillin G. The proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) in an 8% (wt/vol) acrylamide–0.06% (wt/vol) bisacrylamide gel. The labeled PBPs were detected with a tritium storage phosphor screen (GE Healthcare).

Detection of *S. aureus* PBP 2A and *S. sciuri* PBP 4 by Western blotting. Membrane preparations (80 μ g of proteins) were separated by SDS-PAGE as described above. Transfer and blotting were done according to the instructions accompanying the ECL Western blotting analysis system (GE Healthcare). The ChromPure human immunoglobulin G Fc fragment (Jackson ImmunoResearch Laboratories) was used at 3 μ g/ml to eliminate nonspecific hybridization with protein A. The primary antibody was a monoclonal antibody against *S. aureus* PBP 2A (dilution, 1:20,000) obtained by injecting a rabbit with the synthetic peptide sequence CDKNFKQVYKDDSSYISKSDNG conjugated to keyhole limpet hemocyanin (a gift from JoAnn Hoskins, Eli Lilly, Indianapolis, IN), and the secondary antibody was the anti-rabbit antibody included in the kit (dilution, 1:5,000). *S. sciuri* PBP 4 has previously been shown to react also with the monoclonal antibody raised against *S. aureus* PBP 2A (4, 19).

RNA isolation and Northern blot analysis. Total RNA was extracted from cultures grown up to an optical density at 620 nm (OD₆₂₀) of 0.7. RNA (5 μ g) was resolved by electrophoresis on 1.2% agarose–0.66 M formaldehyde gels in morpholinepropanesulfonic acid running buffer. RNA was blotted onto Hy-

bond-N⁺ membranes (GE Healthcare) with a turbo blotter alkaline transfer system (Schleicher & Schuell) with 20 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The PCR-amplified DNA probes were labeled with [α -³²P]dCTP (GE Healthcare) by using a Ready-To-Go labeling kit (GE Healthcare) and hybridized under high-stringency conditions. The blots were subsequently washed and autoradiographed.

Introduction of a plasmid-borne *S. aureus* COL *mecA* gene and *S. sciuri* SS37 *pbpD* gene into *S. aureus* strain COL-S and into a *pbpB* conditional mutant of COL-S. Plasmid pPBP2i (13) was transduced into oxacillin-susceptible *S. aureus* strain COL-S (11) to produce strain COL-S_{spac::pbpB}, in which the expression of the chromosomal *pbpB* gene is under the control of the isopropyl- β -D-1-thiogalactopyranoside (IPTG)-inducible *spac* promoter. A PCR-amplified sequence of the 3,737-bp region of *S. aureus* COL *mecA* was ligated into shuttle plasmid pSPT181C to form pSTSW2C (19). A 4,130-bp fragment that included the promoter region of *pbpD* gene with a complete copy of IS256 inserted 109 bp upstream of the start codon and the entire *pbpD* gene was amplified from *S. sciuri* SS37 by PCR and was cloned into shuttle plasmid pSPT181C to form pSS37MA (20). Recombinant plasmids pSPSW2C and pSS37MA were subsequently introduced into *S. aureus* strain RN4220 by electroporation and were then transduced by phage 80 α into *S. aureus* strain COL-S to yield the transductants COL-S_{AmecA} and COL-S_{SSpbpD}, respectively, and into COL-S_{spac::pbpB} to yield the transductants COL-S_{spac::pbpB/SAmecA} and COL-S_{spac::pbpB/SSpbpD}, respectively.

Cloning of *mecI* and *mecR1* regulatory genes and introduction into *S. aureus*. The 2,466-bp *mecI-mecR1* region from *S. aureus* strain N315 was amplified with primers *mecIP5* (5'-AGAGGGGATCCTCAACGACTTGATTGTTCC-3') and *mecRP9* (5'-GTTCTGAATCTTCTACTTCACCATATATCGC-3') and was ligated into the BamHI and EcoRI sites of high-copy-number plasmid pGC2 (D. C. Oliveira, unpublished data). The resulting recombinant plasmid, pGC2::*mecI-mecR1*, was electroporated into RN4220 and then transduced into COL, COL-S_{AmecA}, and COL-S_{SSpbpD} to produce the transductants COL_{mecI-mecR1}, COL-S_{AmecA/mecI-mecR1}, and COL-S_{SSpbpD/mecI-mecR1}, respectively.

RESULTS

The experimental system: construction of MSSA strain COL-S and introduction of plasmid-borne copies of *S. sciuri* *pbpD* and *S. aureus* *mecA* genes. The chromosomal SCCmec type I cassette was removed from MRSA strain COL by pre-

TABLE 2. Susceptibility to β-lactam antibiotics

Antibiotic	MIC (μg/ml)						
	COL	COL-S	COL-S _{SAmecA}	COL-S _{SSpbpD}	COL _{mecI-mecR1}	COL-S _{SAmecA/mecI-mecR1}	COL-S _{SSpbpD/mecI-mecR1}
Oxacillin	800	0.8	400	400	0.4	0.8	0.4
Ceftizoxime	800	0.8	800	400	3	3	12
Cefotaxime	800	0.8	800	200	6	6	6
Cephadrine	400	6	400	100	6	12	3
Cefoxitin	800	3	400	200	3	6	1.5

cise excision, producing strain COL-S, which was fully susceptible to all beta-lactam antibiotics tested (Table 2) (11). A *pbpB* conditional mutant of strain COL-S (COL-S_{*spac::pbpB*}) was constructed by placing the genetic determinant of PBP 2 under the control of the IPTG-inducible *spac* promoter (13).

The source of the *S. sciuri pbpD* gene was clinical isolate *S. sciuri* SS37, which was recovered from the nasopharynx of a patient (3). Strain SS37 exhibited heterogeneous resistance to oxacillin and carried a *pbpD* gene that was overexpressed due to the insertion of IS256 upstream of the gene (3, 4). The upregulated *pbpD* gene was cloned into a shuttle plasmid (20) and introduced into *S. aureus* COL-S and COL-S_{*spac::pbpB*} to produce transductants COL-S_{SSpbpD} and COL-S_{*spac::pbpB/SSpbpD*}, respectively. As a control, the same shuttle plasmid carrying the *mecA* gene from MRSA strain COL (19) was also introduced into the same *S. aureus* backgrounds, yielding transductants COL-S_{SAmecA} and COL-S_{*spac::pbpB/SAmecA*}, respectively.

Transcription and translation of plasmid-borne *S. aureus mecA* and *S. sciuri pbpD* in *S. aureus*. Figure 1 shows the results of Northern blot analysis, performed to estimate the degree of expression of *S. aureus mecA* and *pbpB* and *S. sciuri pbpD* in the different *S. aureus* backgrounds. No *mecA* transcript was detected in COL-S or its *pbpB* conditional mutant, COL-S_{*spac::pbpB*}. *S. aureus mecA* and *S. sciuri pbpD* were both effectively and highly expressed in transductants COL-S_{*spac::pbpB/SAmecA*} and COL-S_{*spac::pbpB/SSpbpD*}. The inhibition of *pbpB* transcription was confirmed in cultures of strains COL-

S_{*spac::pbpB/SAmecA*} and COL-S_{*spac::pbpB/SSpbpD*} grown in the absence of the IPTG inducer.

The PBP patterns were determined by incubating membrane preparations in the presence of radioactive penicillin (Fig. 2A),

FIG. 1. Transcription of *S. aureus mecA* and *pbpB* genes and *S. sciuri pbpD* gene. RNA was purified from cultures grown at 30°C to an OD₆₂₀ of 0.7. RNA (5 μg) was resolved by electrophoresis on agarose-formaldehyde gels. After transfer, the membranes were hybridized with ³²P-labeled *S. aureus mecA* and *pbpB* and *S. sciuri pbpD* DNA probes.

FIG. 2. PBP patterns and detection of protein products of *S. aureus mecA* (PBP 2A) and *S. sciuri pbpD* (PBP 4). (A) Membrane preparations (150 μg of proteins) were incubated with a single saturating concentration of [¹⁴C]benzylpenicillin. After SDS-PAGE, the gel was exposed to a tritium storage phosphor screen for 2 weeks. (B) Membrane preparations (80 μg of proteins) were tested by Western blot analysis for the production of proteins that react with a monoclonal antibody raised against *S. aureus* PBP 2A. The results for *S. aureus* COL and *S. sciuri* SS37 are provided as positive controls and size markers for PBP 2A and PBP 4, respectively. (C) Protein patterns on an SDS-polyacrylamide gel stained with Coomassie blue.

FIG. 3. Effect of suppression of *pbpB* transcription and introduction of the plasmid-borne *S. aureus mecA* or *S. sciuri pbpD* gene on growth in *S. aureus*. Overnight cultures supplemented with IPTG were centrifuged, washed twice, and suspended in fresh medium to an initial OD_{620} of 0.01 in the presence of 500 μ M IPTG (closed symbols and solid lines) or in the absence of IPTG (open symbols and dashed lines). The turbidities of the cultures were monitored at 30°C for 12 h. (A) COL-*S*_{spac::pbpB} (■ and □) and COL-*S*_{spac::pbpB} (▲ and △); (B) COL-*S*_{spac::pbpB/SAmecA} (● and ○) and COL-*S*_{spac::pbpB/SSpbpD} (◆ and ◇).

and a Western blot analysis was performed with an antibody raised against *S. aureus* PBP 2A (Fig. 2B), which has previously been shown to also react with *S. sciuri* PBP 4 (4, 19, 20). In accordance with the results of the Northern blot analysis, these experiments confirmed that PBP 2 was absent from the membrane preparation of COL-*S*_{spac::pbpB/SSpbpD} grown in the absence of IPTG and that PBP 2A was absent from COL-*S* and its derivative, COL-*S*_{spac::pbpB}. Large amounts of PBP 2A were detected in the membrane preparations of COL-*S*_{spac::pbpB/SAmecA}. The foreign *S. sciuri* PBP 4 was also correctly translocated and deposited in large amounts in the plasma membrane of *S. aureus* host cells. As shown in Fig. 2C, *S. aureus* PBP 2A (78 kDa) and *S. sciuri* PBP 4 (84 kDa) were already evident from the SDS-polyacrylamide gel stained with Coomassie blue.

Similarly, high levels of transcription of *S. aureus mecA* and *S. sciuri pbpD* and large amounts of PBP 2 and PBP 4 were also observed for transductants COL-*S*_{SAmecA} and COL-*S*_{SSpbpD}, respectively (data not shown).

Phenotypic expression of β -lactam resistance. Table 2 shows that the introduction of both the *S. sciuri pbpD* gene and the *S. aureus mecA* gene into MSSA strain COL-*S* was able to provide virtually identical levels of resistance to a group of β -lactam antibiotics which have considerably different degrees of selective binding to the four *S. aureus* PBPs. Transductants COL-*S*_{SSpbpD} and COL-*S*_{SAmecA} produced high-level and homogeneous resistance to ceftizoxime and cefotaxime (selective affinity for PBP 2), cephradine (PBP 3), cefoxitin (PBP 4), and oxacillin.

Inhibition of β -lactam resistance in transductants COL-*S*_{SSpbpD} and COL-*S*_{SAmecA} by subinhibitory concentrations of D-cycloserine and by the *mecI-mecR1* regulatory genes. It has previously been shown that inhibitors of the early steps of cell wall synthesis can reduce the level of methicillin resistance and change the homogeneous resistance phenotype of strain COL to a heterogeneous one (16). Susceptibility to oxacillin in the presence of subinhibitory concentrations ($0.25 \times$ MIC) of the cell wall synthesis inhibitor D-cycloserine was determined by population analysis. While the oxacillin MIC of strain COL-*S*

remained unchanged, the resistance levels were drastically reduced in the presence of D-cycloserine, from MICs of 800 to 400 μ g/ml for strains COL, COL-*S*_{SAmecA}, and COL-*S*_{SSpbpD} to MICs as low as 1, 8, and 0.75 μ g/ml, respectively. In addition, the homogeneous expression of oxacillin resistance in COL-*S*_{SAmecA} and COL-*S*_{SSpbpD} was converted to heterogeneous expression (data not shown), as previously described for COL (16).

The *S. aureus mecA* gene has been shown to be transcriptionally regulated in some clinical isolates by *mecR1* and *mecI*, cotranscribed chromosomal genes that encode a signal transducer and a repressor, respectively (9). The *mecI-mecR1* region from *S. aureus* strain N315 was cloned in a high-copy-number plasmid and introduced into COL, COL-*S*_{SAmecA}, and COL-*S*_{SSpbpD}. The *mecI* and *mecR1* regulatory genes repressed the phenotypic expression of β -lactam resistance in COL, as described previously (10), and well as in COL-*S*_{SAmecA} (Table 2). Most interestingly, the level of β -lactam resistance was also extensively repressed in strain COL-*S*_{SSpbpD}, which expressed the plasmid-borne *S. sciuri pbpD* gene (Table 2), suggesting that the MecI repressor can bind to the promoter region of *S. sciuri pbpD* similar to the way in which it binds to the promoter region of *S. aureus mecA* (14).

Replacement of the normal (essential) physiological function(s) of the *S. aureus* host PBP 2 by *S. sciuri* PBP 4. Previous studies have shown that *S. aureus* PBP 2, the protein product of the *pbpB* gene, is essential for growth in β -lactam-susceptible *S. aureus* strains. However, the additional PBP 2A present in MRSA strains can replace the essential function(s) of PBP 2 (7, 13). In order to test if *S. sciuri* PBP 4 was also able to support growth in PBP 2-deprived *S. aureus* cells, the plasmid-borne *S. sciuri pbpD* gene was introduced into *S. aureus* strain COL-*S*_{spac::pbpB}, in which the *pbpB* gene was put under the control of an IPTG-inducible promoter. As a control, the plasmid-borne *S. aureus mecA* gene was also introduced in the same background. As previously described for a β -lactam-susceptible *S. aureus* strain, the absence of IPTG prevented the growth of COL-*S*_{spac::pbpB} but only slowed the growth of the *pbpB* conditional mutant of MRSA strain COL, COL-*S*_{spac::pbpB}

FIG. 4. HPLC elution profiles of purified peptidoglycans. Peptidoglycans were purified from 1-liter cultures grown at 30°C to an OD_{620} of 0.4, digested with mutanolysin, and separated by HPLC. The peptidoglycan compositions of *S. aureus* COL (A), transductants COL-S_{spac::pbpB}/SAmecA (B) and COL-S_{spac::pbpB}/SSpbpD (C) grown in the absence of IPTG, and *S. sciuri* SS37 (D) were determined. Muropeptides were detected by measurement of the absorbance at 206 nm.

(Fig. 3A). The introduction of the plasmid-borne *S. sciuri* *pbpD* gene into COL-S_{spac::pbpB} allowed the growth of strain COL-S_{spac::pbpB}/SSpbpD in the absence of IPTG, and the growth rate was comparable to that of COL-S_{spac::pbpB}/SAmecA carrying the plasmid-borne *S. aureus* *mecA* gene (Fig. 3B) indicating that, similarly to *S. aureus* PBP 2A, *S. sciuri* PBP 4 can replace the essential function(s) of the native PBP 2 of *S. aureus*.

Effect of suppression of *pbpB* transcription on peptidoglycan composition of transductants carrying the plasmid-borne *S. sciuri* *pbpD* or *S. aureus* *mecA* gene. Peptidoglycans were puri-

FIG. 5. Effect of introduction of the plasmid-borne *S. aureus* *mecA* or *S. sciuri* *pbpD* genes and suppression of *pbpB* transcription on the expression of oxacillin resistance in *S. aureus*. Aliquots of overnight cultures were plated on tryptic soy agar containing increasing concentrations of oxacillin. The numbers of CFU were counted after incubation for 72 h at 30°C. The oxacillin susceptibility profiles were determined for COL (◆), COL-S_{spac::pbpB} grown in the presence (▲) and absence (Δ) of IPTG, COL-S_{spac::pbpB}/SAmecA grown in the presence (▼) and absence (●) of IPTG, and COL-S_{spac::pbpB}/SSpbpD grown in the presence (■) and absence (□) of IPTG.

fied from MRSA strain COL and from the two transductants COL-S_{spac::pbpB}/SAmecA and COL-S_{spac::pbpB}/SSpbpD carrying the plasmid-borne *S. aureus* *mecA* and *S. sciuri* *pbpD* genes, respectively, in the background of the *pbpB* conditional mutant of COL-S grown in the absence of IPTG. Under these conditions of growth (the absence of IPTG), the plasmid-borne *S. aureus* *mecA* and *S. sciuri* *pbpD* genes provide the normal (essential) function(s) of the native PBP 2 of *S. aureus*. The muropeptide compositions of the peptidoglycans were analyzed by HPLC (Fig. 4). The suppression of *pbpB* transcription in cultures of COL-S_{spac::pbpB}/SAmecA (Fig. 4B) resulted in the production of peptidoglycan with a moderate decrease in the relative proportion of the highly cross-linked muropeptides with retention times of over 100 min and a parallel slight increase in the proportion of the monomeric muropeptide 5 compared to the peptidoglycan composition of COL as shown in Fig. 4A. Similar variations in the peptidoglycan composition were observed in cultures of strain COL in which *pbpB* transcription was suppressed (7). The HPLC elution profile of COL-S_{spac::pbpB}/SSpbpD grown in the absence of IPTG (Fig. 4C) clearly differed from that of *S. sciuri* strain SS37 (Fig. 4D) but was identical to that of COL-S_{spac::pbpB}/SAmecA. These results indicate that *S. sciuri* PBP 4 was able to replace the essential enzymatic activity of *S. aureus* PBP 2 and catalyze the biosynthesis of peptidoglycan to produce an *S. aureus* type of cell wall with the cell wall precursors of the *S. aureus* host.

Effect of suppression of *pbpB* transcription on the oxacillin resistance level of transductants carrying the plasmid-borne *S. sciuri* *pbpD* or *S. aureus* *mecA* gene. High-level and homogeneous resistance to oxacillin in MRSA strain COL is known to depend on the level of transcription of the *S. aureus* *pbpB* gene (7). As shown in Fig. 5, the inhibition of *pbpB* transcription in the conditional mutant of strain COL (COL-S_{spac::pbpB}) reduced the oxacillin resistance levels of the majority of the cells and converted the homogeneous phenotype to a heterogeneous one. In the presence of IPTG, the introduction of either the

plasmid-borne *S. sciuri pbpD* gene or the *S. aureus mecA* gene into the *pbpB* conditional mutant COL-*S_{spac::pbpB}* produced high-level and homogeneous resistance that was close to that of *S. aureus* strain COL (Fig. 5). However, in the absence of IPTG, the oxacillin MICs for the majority of the cells decreased 16-fold and the population analysis profiles became heterogeneous (Fig. 5). These results indicate that even in the presence of large amounts of either *S. sciuri* PBP 4 or *S. aureus* PBP 2A in the transductants, the host PBP 2 was still required for the optimal expression of antibiotic resistance in *S. aureus*, as was already shown for MRSA strain COL (7, 12).

DISCUSSION

The genetic determinant of β -lactam resistance, *mecA*, is not native to *S. aureus* but was acquired from an extraspecies source (1). The *S. sciuri pbpD* gene, which is uniformly present in both β -lactam-susceptible and -resistant isolates of this widely spread animal commensal species, was first identified on the basis of its high degree of structural similarity with the *S. aureus mecA* gene (2, 18). Previous studies have proposed that *S. sciuri pbpD* may represent the evolutionary precursor of the *S. aureus mecA* gene, mainly on the basis of epidemiological and genetic evidence. The protein product of *pbpD* was subsequently identified as PBP 4, one of the six PBPs detected in *S. sciuri* (20). Recombinant *S. sciuri* PBP 4 was purified and was shown to share several biochemical properties with *S. aureus* PBP 2A (6).

In this report, a new experimental system was designed to further test the validity of the proposition that the *mecA* resistance determinant present in all MRSA strains may have originated from the *S. sciuri pbpD* gene. The resistance cassette *SCCmec* was excised from extensively studied MRSA strain COL to generate MSSA strain COL-S, which was subsequently used as the recipient of a plasmid-borne copy of an upregulated form of *S. sciuri pbpD* recovered from resistant *S. sciuri* clinical isolate SS37.

The transductants carrying the *S. sciuri pbpD* gene exhibited most, if not all, of the resistance-related properties of the original MRSA strain COL.

(i) *S. aureus* cells were able to transcribe and translate this foreign genetic determinant producing large amounts of *S. sciuri* PBP 4, which was deposited in the plasma membrane of the host bacterium. (ii) *S. sciuri pbpD* could function as an effective component of the antibiotic resistance mechanism in the heterologous background of *S. aureus*. The transductants had extremely high-level, homogeneous, and broad-spectrum resistance to structurally diverse β -lactam antibiotics, similar to the properties of MRSA strain COL. Also, similarly to strain COL, (iii) the oxacillin resistance level of the transductants was dependent on the function of the host *S. aureus* PBP 2 (7) and (iv) oxacillin resistance was repressed by inhibitors of an early step in cell wall biosynthesis (16) and by the specific regulatory genes *mecI* and *mecR* (10), suggesting that the *MecI* repressor can bind to the promoter region of the *S. sciuri pbpD* gene. (v) *S. sciuri* PBP 4 was able to fully replace the essential physiological function(s) of the native *S. aureus* PBP 2 in a conditional mutant in which transcription of *pbpB* gene was inhibited (7, 13). (vi) The composition of the cell wall peptidoglycan produced under these conditions was typical of that of the *S.*

aureus peptidoglycan (5), indicating that *S. sciuri* PBP 4 can function as a cell wall synthetic enzyme in *S. aureus* and cooperate with the other PBPs and monofunctional transglycosylases of the host to build cell wall peptidoglycan.

Thus, by replacing the *S. aureus* chromosomal determinant of resistance to β -lactams with a plasmid-borne copy of the heterologous upregulated *S. sciuri pbpD* gene, we were able to reconstruct a typical MRSA strain which was phenotypically indistinguishable from original MRSA strain COL. These results provide further support for the proposition that the wide-spectrum β -lactam resistance determinant *mecA* carried by all MRSA strains has evolved from the *S. sciuri pbpD* gene.

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